High-throughput screening to identify novel inhibitors of human α-methylacyl-CoA racemase 1A (AMACR; P504S)

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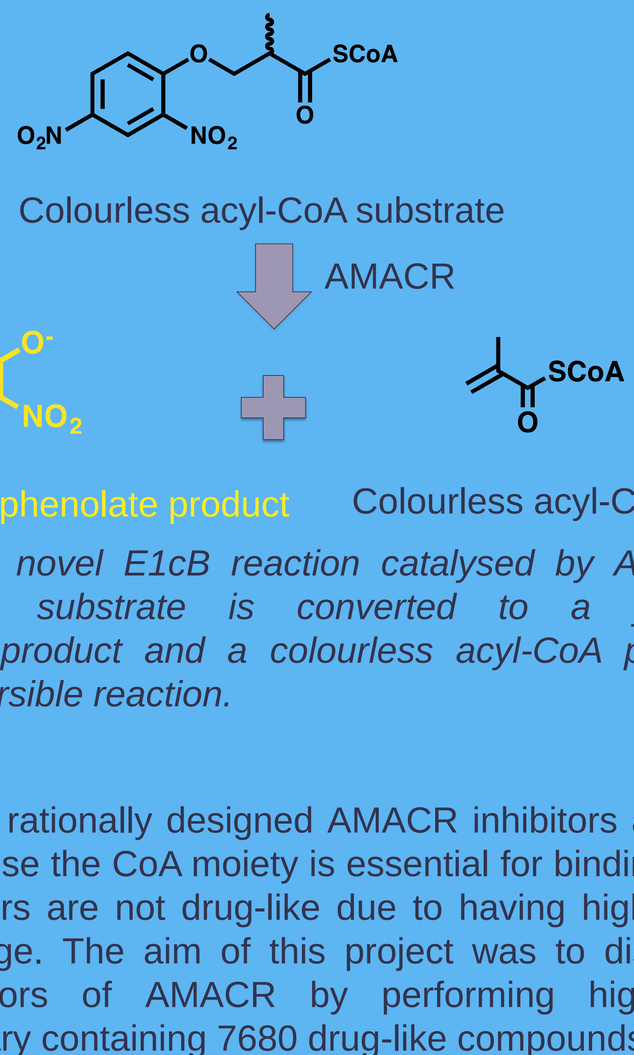
Introduction

Phytanic acid [3*R*,*S*,7*R*,11*R*-(3,7,11,15-tetramethyl)hexadecanoic acid] is a dietary fatty acid, particularly abundant in foods such as red meat and dairy products.[1] Phytanic acid is metabolised by α-oxidation in the body to produce a mixture of 2*R*- and 2*S*-methyl pristanic acid [2*R*,*S*,6*R*,10*R*-(2,6,10,14-tetramethyl)pentadecanoic acid] epimers.[2] The acyl-CoA oxidases responsible for the further metabolism of pristanoyl-CoA by β-oxidation have the absolute requirement for the 2*S*-methyl configuration.[3] The enzyme involved in the conversion of the 2*R* to the 2*S* isomer is α-methylacyl-CoA racemase (AMACR).[4]

AMACR levels are increased (up to 9-fold) in all types of prostate cancers and phytanic acid has been shown to have a role in regulating levels.[5] It was demonstrated that reducing AMACR levels using siRNA reduced the proliferation of prostate cancer cells, making AMACR an attractive target.[6]

A recently developed colorimetric assay uses a reaction, in which a colourless acyl-CoA substrate is converted into a yellow product by AMACR (Scheme 1). The formation of the yellow product allows the activity of the AMACR to be assayed in 96-well plates allowing simultaneous analysis of many samples (unpublished work, paper in preparation).



Scheme 1: *The novel E1cB reaction catalysed by AMACR. The colourless CoA substrate is converted to a yellow 2,4-dinitrophenolate product and a colourless acyl-CoA product in a single-step irreversible reaction.*

Project Aims

Currently, all the rationally designed AMACR inhibitors are acyl-CoA analogues because the CoA moiety is essential for binding. However, acyl-CoA inhibitors are not drug-like due to having high MW and a zwitterionic charge. The aim of this project was to discover small molecule inhibitors of AMACR by performing high-throughput screening of library containing 7680 drug-like compounds.

Methods

High throughput screening was performed in 96-well half area plates in 100μL total volume. The activity of the enzyme was assayed by measuring the absorbance at 354 nm and 390 nm. The final concentration of library compounds, substrate and enzyme were 30 μM, 18 μM and 0.087 mg.mL^{-1}.

Figure 1: *High-throughput screening of library compounds.*

Results and Discussion

Human His-tagged enzyme was expressed in Rosetta2 (DE3) cells and lysed using the One Shot cell disruption system. The enzyme was purified by metal chelate chromatography and buffer exchanged in 10 mM NaH_2PO_4-NaOH, pH 7.4 (Figure 2).

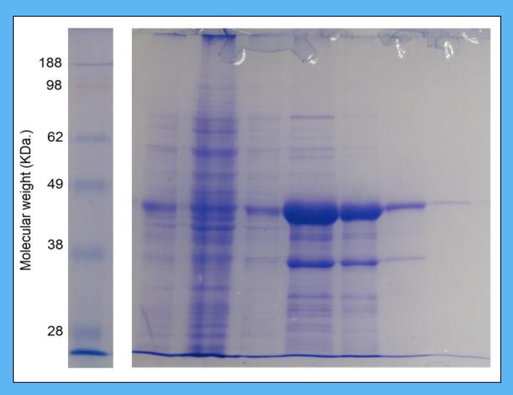


Figure 2: *SDS-PAGE analysis of human AMACR 1A.*

High throughput screening

Compounds were screened using the assay and possible inhibitors (‘hits’) were identified by comparing absorbance traces with those for positive and negative controls, Approximately 70 ‘hits’ were identified, giving a ‘hit’ rate of ~0.9%, which is consistent with the hit rate of <1% expected from a diverse and unbiased library.

IC_{50} Determination

IC_{50} values for some known AMACR inhibitors, such as *R*- and *S*-Ibuprofenoyl-CoA, ranged between 0.5 μM and 20 μM.[7,8] IC_{50} values for the new compounds were determined (Figure 3), which were in the same range. This suggests that the new compounds have a similar potency to rationally designed inhibitors.

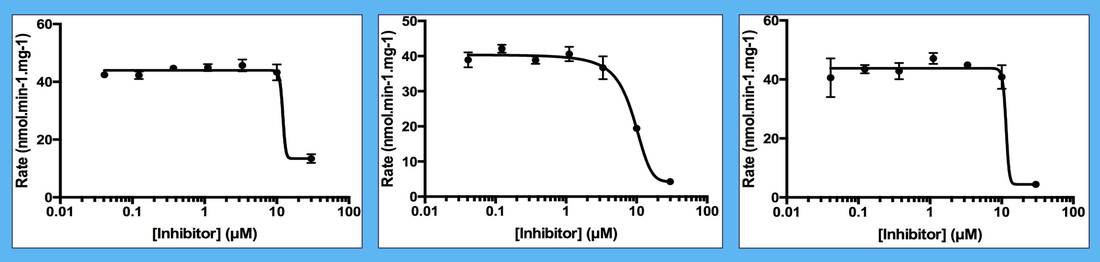


Figure 3: *IC_{50} curves for three selected inhibitors (drugs 1-3). The determined IC_{50} values were: 15.2 μM (Drug 1) 9.3 μM (Drug 2) and 12.8 μM (Drug 3).*

Reversibility experiments

Reversibility experiments also suggest that the compounds identified are reversible enzyme inhibitors as opposing to non-specific denaturing agents (Figure 4). The mode of inhibition is reversible because the activity of the enzyme is fully restored upon diluting the 30 μM inhibitor to 0.66 μM inhibitor.

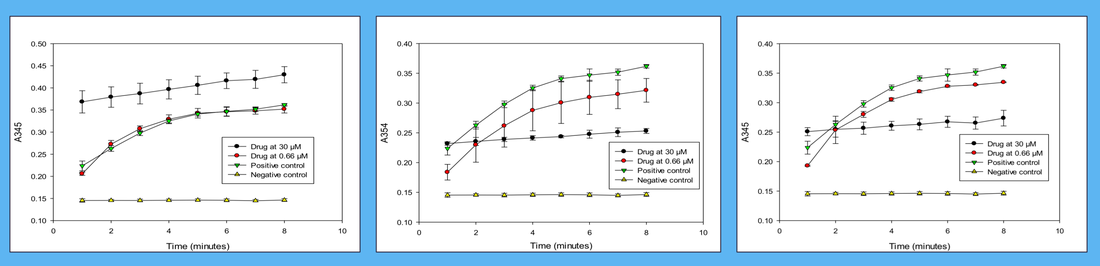


Figure 4: *Reversibility experiments three selected inhibitors (drugs 1-3).*

Acknowledgements

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References

1. Lloyd*, et al.*, *Prog. Lipid Res.*, **2013**, 52, 220-230
2. Ackman, *et al., Lipids,* **1967**, 2, 357-62
3. Battaile, *et al., Lipid Metab.,* **1998**, 1390, 333-8
4. Schmitz, *et al., Eur. J. Biohem.,* **1995**, 231, 815-22
5. Zha*, et al.*, *Cancer Res.*, **2002**, 62, 2220-2226
6. Wright, *et al., Prostate,* **2011**, 71, 498-506
7. Carnell, *et al., J. Med. Chem.,* **2007**, 50, 2700-2707
8. Carnell, *et al., ChemMedChem.,* **2013**, 8, 1643-1647